THE DESTRUCTION OF ASCARIS EGGS

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Most investigators are agreed that Ascaris eggs have strong powers of resistance to chemical fluids of various kinds. I also have tested the resistance of Ascaris eggs containing embryos by soaking them in various disinfectants, acid or alkaline, and have found that in every experiment they retained for many days the ability to infect.

I found, however, that Ascaris eggs were easily destroyed by heat. In September, 1921, I poured hot water at a temperature of 100° C. over Ascaris eggs collected from human dejecta and found that the eggs lost their power of development. In November of the same year I noticed that Ascaris eggs containing embryos, after hot water had been poured on them, lost the ability to infect.

Having ascertained that Ascaris eggs were instantly destroyed by water at 100° C., I attempted to discover how long they would remain alive in water at varying degrees of temperature. These tests, however, failed on account of the difficulty of finding any effective means of immersing in hot water Ascaris eggs dispersed among human dejecta. Again, if eggs mingled with human dejecta are dispersed in boiling water, it is not always possible to get them out at once.

At length, a method was discovered which seemed to me entirely effective. The following experiments were than carried out.

Method : In testing the resistance of Ascaris eggs to heat, I employed matches in order to prevent the dispersal of the eggs. The human dejecta were washed in water and the sediment obtained by centrifuging was smeared on the matches to half their length, and left to dry. Then each match, held with the forceps, was soaked for the desired period in hot water at varying temperatures. They were then put into cold water to cool. The matches were then subjected to observation.

The observations were carried out in three ways, (1) staining ; (2) development ; (3) infection.

(1) I use Sudan III staining for the purpose of distinguishing between live and dead Ascaris eggs. Presuming that fatty

degeneration might possibly occur in the liver of a patient infected with Ascaris, we applied Sudan III staining to the liver of an animal which was experimentally infected with Ascaris. We found that Ascaris larvae in the liver were distinctly stained by Sudan III staining, and that living larvae separated from the liver or lungs could be similarly stained. The red-stained granules of 'fat-corpuscles,' as I provisionally call them, gradually decrease in size as the size of the larva increases at each stage of its development.

With the object of making clear the true nature of the ' fat-corpuscles ' of the Ascaris larva, I tried to ascertain if there were any substance in Ascaris eggs which could be stained with Sudan III. It was necessary to make sections for the staining of the eggs. The Ascaris eggs, collected from human dejecta, were embedded in gelatine and cut into thin sections with the freezing microtome. They were then stained with Sudan III. By this means the staining of eggs was obtained, but not of the albuminous membrane. I further noticed that fat-corpuscles were abundant in the eggs.

When I applied Sudan staining to Ascaris eggs collected from human dejecta, I found that unfertilised eggs were stained with red granular spots, while healthy fertilised eggs were not stained at all. Therefore, presuming that Ascaris eggs might be made stainable by killing them, I applied Sudan III staining to eggs killed by boiling water. As I expected, all the eggs were seen under the microscope with fine red colouring.

From the results of my repeated experiments, I conclude that the fat-corpuscles are produced in the blastomeres as the egg-cells grow and segment, and that gradually these fat-corpuscles gather much more in the hypoblastic than in the epiblastic cells ; thus very young U-shaped embryos are full of fat-corpuscles from head to tail ; but these fat-corpuscles are ranged along the intestine of the worms as they grow. We consider, therefore, that the above-mentioned fat-corpuscles may be the yolk.

Having found, therefore, that (*a*) healthy fertilised eggs are unstainable with Sudan III, that (*b*) unfertilised eggs are stainable, and that (*c*) fertilised eggs become stainable with that dye if hot water is poured over them, we used Sudan III for the study of Ascaris eggs after immersion in hot water at temperatures varying from 40° C. to boiling point, and for periods varying from one second

to an hour. We found that Ascaris eggs become stainable with Sudan III after being immersed for one second in water at over 75° C., almost all become stainable after being immersed for ten seconds in water at 70° C., while in water at 65° C. they had to be immersed for over ten minutes before becoming stainable. In water at temperatures lower than 60° C. over one hour's immersion is not sufficient to render them stainable.

The above experiment was repeated twenty times with material from the same patient and from twenty-one others, but the results showed no great difference.

(2) In order to prove that Ascaris eggs which have become stainable are really dead, we cultivated, in 4 per cent. formalin solution, Ascaris eggs which had been immersed in water at varying temperatures for varying periods of time as in Experiment 1, and examined their developmental condition at the end of stated periods. This was repeated some thirty times with material from the same patient and from many others, but all the results were practically the same, namely, that all the eggs lost their power of development after being immersed in water at over 70° C. for one second; a few retained it after being dipped in water at 65° C. for one second; embryos developed from all eggs immersed for three seconds in water at 60° C., for 40 seconds in water at 55° C., and for thirty minutes in water at 50° C.; all eggs would develop into embryos after being soaked for one hour in water at temperatures lower than 45° C.

The difference between the results of Experiments 1 and 2 is noteworthy.

Ascaris eggs which have lost their power of development can be divided into two groups, one stainable with Sudan III, the other unstainable with that dye. The former show a morphological change (i.e., fatty degeneration and distortion of the eggs) after immersion in hot water; the latter do not differ from healthy eggs, and yet are in a state of suspended development. This condition of suspended development may continue for as long as twenty days or even longer, but if left alone for long they are likely to perish gradually.

Ascaris eggs are instantly killed by very hot water; by water at lower temperatures they are merely deprived of their ability to develop; and after being immersed in water at temperatures below 45° C. the eggs develop in the usual manner.

(3) As a result of Experiments 1 and 2 I could definitely fix the temperature at which hot water will destroy Ascaris eggs containing embryos capable of infection.

I cultivated for a month, in 4 per cent. formalin solution, Ascaris eggs collected from human dejecta. When matured, I placed them on matches and left them to dry until the following day. I then soaked these matches in hot water at various temperatures and fed mice with the eggs. After three days I examined the mice with a view to ascertaining whether liver, heart, or lungs were infected. I repeated this experiment many times with 515 mice and arrived finally at the following results.

The mature Ascaris eggs, cultivated in 4 per cent. formalin solution, lose their infecting power after remaining for one second in water at 70° C. or more ; almost all lose it after remaining for one second in water at 65° C., for five seconds in water at 60° C., for forty seconds in water at 55° C., or for fifteen minutes in water at 50° C. Even after remaining for one hour, however, in water at temperatures below 45° C. the eggs did not lose their power to infect.

SUMMARY

A minute examination of the power of resistance to heat of Ascaris eggs by means of the above experiments has led to the conclusion that the method of destruction of Ascaris eggs by boiling water can be effected also with hot water at lower temperatures. Generally speaking, the ideal to be aimed at in disinfection is simplicity of method and rapidity of action.

To prevent Ascaris infection it is safest to kill the eggs by immersion in hot water at the temperature at which the egg content changes morphologically and becomes stainable with Sudan III ; though the power of development and the power to infect may be destroyed by immersion in water at over 70° C. for one second, at 65° C. for two seconds, at 60° C. for five seconds, at 55° C. for fifty seconds and at 50° C. for forty-five minutes.

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